Oncogene (2000) 19, 968–988

www.nature.com/onc

The mammary pathology of genetically engineered mice: the consensus report and recommendations from the Annapolis meeting‡

Robert D Cardiff*[,1,]†, Miriam R Anver[2], Barry A Gusterson[3], Lothar Hennighausen[4], Roy A Jensen[5], Maria J Merino[6], Sabine Rehm[7], Jose Russo[8], Fattaneh A Tavassoli[9], Lalage M Wakefield[10] Jerrold M Ward[11] and Jeffrey E Green[10,]†

[1]U.C.D. Center for Comparative Medicine, County Road 98 and Hutchison Drive, University of California, Davis, Davis, CA 95616, USA; [2]Pathology/Histotechnology Laboratory, SAIC Frederick, NCI-FCRDC, P.O. Box B, Building 539, Frederick, MD 21702-1201, USA; [3]Institute of Cancer Research, The Breakthrough Toby Robins Breast Cancer Research Centre, 237 Fulham Road, London SW3 6JB, England; [4]Laboratory of Genetics and Physiology, NIDDK, NIH, Building 8, Room 101, Bethesda, MD 20892, USA; [5]Department of Pathology, 4918 TVC, Vanderbilt University Medical Center, 22nd Avenue South and Pierce Avenue, Nashville, TN 37232-5310, USA; [6]Laboratory of Pathology, NCI, NIH, Building 10, Room 2N212, Bethesda, MD 20892, USA; [7]SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA, USA; [8]Breast Cancer Research Laboratory, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA; [9]Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306-6000, USA; [10]Laboratory of Cell Regulation and Carcinogenesis, NCI, NIH, Building 41, Room C629, 41 Library Drive, Bethesda, MD 20892, USA; [11]Veterinary and Tumor Pathology Section, Office of Laboratory Animal Resources, National Cancer Institute, Frederick, MD 21702-1201, USA

NIH sponsored a meeting of medical and veterinary pathologists with mammary gland expertise in Annapolis in March 1999. Rapid development of mouse mammary models has accentuated the need for definitions of the mammary lesions in genetically engineered mice (GEM) and to assess their usefulness as models of human breast disease. The panel of nine pathologists independently reviewed material representing over 90% of the published systems. The GEM tumors were found to have: (1) phenotypes similar to those of non-GEM; (2) signature phenotypes specific to the transgene; and (3) some morphological similarities to the human disease. The current mouse mammary and human breast tumor classifications describe the majority of GEM lesions but unique morphologic lesions are found in many GEM. Since little information is available on the natural history of GEM lesions, a simple morphologic nomenclature is proposed that allows direct comparisons between models. Future progress requires rigorous application of guidelines covering pathologic examination of the mammary gland and the whole animal. Since the phenotype of the lesions is an essential component of their molecular pathology, funding agencies should adopt policies ensuring careful morphological evaluation of any funded research involving animal models. A pathologist should be part of each research team. *Oncogene* (2000) **19,** 968–988.

Keywords: genetically engineered mice; mouse mammary models; breast tumor classifications

*Correspondence: RD Cardiff
†RD Cardiff assisted in the organization of the pathology workshop and prepared the manuscript. JE Green was primarily responsible for the workshop concept and organization. All other authors contributed equally to the manuscript and are, thus, listed alphabetically
‡The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the US Government

Introduction

Genetic engineering of mice for mammary biology is now in its second decade. Almost 100 transgenes, targeted mutations (site-directed mutations, knock outs and knock ins), combinations of transgenes and combinations of transgenes and targeted mutations have been used to study mammary cancer in mice. These mice will be referred to here under the collective term of Genetically Engineered Mice (GEM). Surprisingly few reports provide detailed analysis of the tumor pathology or the natural history and evolution of neoplastic progression. Although many investigators have claimed that their animals are models for human breast cancer, few have actually validated their models and provided direct comparisons with human tissues.

The National Institutes of Health (NIH) Breast Cancer Think Tank (BCTT), an internal organization responsible for determining key issues in breast cancer, identified that the accurate pathologic analysis of lesions in genetically engineered mice is a key to progress in understanding these models. On March 3–5, 1999, a panel of nine surgical, veterinary and experimental pathologists was convened in Annapolis Maryland, sponsored by the NIH, BCTT and the NCI Cancer Genome Anatomy Project (CGAP). The Panel was asked to compare the pathology and classification of mouse and human breast cancers. The following report includes the considerations and recommendations of the Annapolis Pathology Panel and its organizers.

Materials and methods

A set of 175 slides representing 39 models of genetically engineered mice was developed through the generous donation of blocks and slides by investigators from around the world (summarized in Table 1). In addition, two models of transgenesis in rats, three transplant models, two models of chemical carcinogenesis and three examples of Mouse Mammary

Table 1 Summary of models

Models	*Transgene*	*Bitransgene/ exptl. manipulation*	*Species*	*Promotors*	*References*
Growth factors	FGF3 (INT2)	WNT1	Mouse	MMTV-LTR	(Kwan, *et al*., 1992; Muller *et al*., 1990)
	FGF7 (KGF)		Mouse	MMTV-LTR	(Kitsberg and Leder, 1996)
	HEREGULIN(NDF)	myc	Mouse	MMTV-LTR	(Krane and Leder, 1996)
	HGF		Mouse	MT	(Takayama *et al*., 1997)
	IGFII		Mouse	BGL, H19	(Bates *et al*., 1995; Pravtcheve and Wise, 1998)
	TGFα	p53-172H, myc, DMBA	Mouse	WAP, MMTV-LTR, MT	(Coffey *et al*., 1994; Jhappan *et al*., 1990; Matsui *et al*., 1990; Sandgren *et al*., 1990, 1995)
	TGF-β	MMTV-infected	Mouse	WAP	(Korden *et al*., 1995)
Receptors	TGFβ DNIIR		Mouse	MMTV-LTR	(Joseph *et al*., 1999)
	ERB-B2 (neu)	p53-172H	Mouse	MMTV-LTR	(Guy *et al*., 1992b; Li *et al*., 1997, Muller *et al*., 1988)
	RET-1		Mouse	MMTV-LTR	(Iwamoto *et al*., 1990)
	Tpr-MET		Mouse	MMTV-LTR	(Liang *et al*., 1996)
Signal pathways	PyV-mT		Mouse	MMTV-LTR	(Guy *et al*., 1992a)
	RAS		Mouse	MMTV-LTR	(Sinn *et al*., 1987)
Cell cycle	CYCLIN D1		Mouse	MMTV-LTR	(Wang *et al*., 1984)
	MYC	BCL-2	Mouse	MMTV-LTR, WAP	(Jager *et al*., 1997, Stewart *et al*., 1984)
	p53-172H, p53		Mouse	WAP, Null	(Donehower *et al*., 1992; Li *et al*., 1997)
	SV40TAg	BCL-2	Mouse	WAP, C(3)1	(Furth *et al*., 1999; Husler *et al*., 1998; Maroulakou *et al*., 1994; Tzeng *et al*., 1993)
Differentiation	NOTCH4(INT3)	TGFβ	Mouse	MMTV-LTR, WAP	(Gallahan *et al*., 1996; Smith *et al*., 1995)
	WNT 1		Mouse	MMTV-LTR	(Tsukamoto *et al*., 1988)
	WNT10b		Mouse	MMTV-LTR	(Lane and Leder, 1997)
	P-CADHERIN		Mouse	Null	(Radice *et al*., 1997)
Other Transgenes	Stromelysin		Mouse	WAP	(Sternlicht *et al*., 1999)
	MTS1		Mouse	MMTV-LTR	(Ambartsumian *et al*., 1996)
Transgenic Rat Models	TGF α		Rat	MMTV-LTR	(Davies *et al*., 1999)
	SV40Tag		Rat	C(3)1	
	ERB-B2 (neu)		Rat	MMTV-LTR	(Davies *et al*., 1999) (Shibata *et al*, 1999)
Miscellaneous	DMBA treated rat		Rat		(Russo *et al*., 1977)
	MNU treated rat		Rat		(Anzano *et al*., 1994)
	MMTV infected mouse		Mouse		(Rehm and Liebelt, 1996)
	Transplants of DMBA treated-p53$^{-/-}$ mouse mammary epithelium		Mouse		(Jerry *et al*., 1999)

Tumor Virus (MMTV)-induced tumors were available for comparison. The slide set was developed, distributed and studied in advance of the meeting in Annapolis. The available experimental and demographic data was also provided for each model. In addition, the members of the Panel shared published and unpublished cases from their own archives that included their experience with over 50 additional model systems. The slide set was a unique opportunity to compare the histopathology of the majority of existing models.

Many of the biologists who created the GEM models attended the Annapolis meeting to present and discuss the biology of the individual models. The key publications were listed and available for the Panel. Special stains of selected cases were performed and available to the Panel for additional study.

Following the Annapolis meeting, the Pathology Panel continued their studies and deliberations, meeting for a final review on July 26 and 27, 1999 at NIH, Bethesda, MD. The final draft of this report was subsequently developed. This paper represents the collective views of the Panel.

Nomenclature

Historical background

A number of classification schemes for both human and mouse mammary cancer were reviewed. Existing classification schemes for mouse mammary tumors were developed to describe tumors that arose ‘spontaneously’ (usually induced by mouse mammary tumor virus (MMTV)), or were induced by chemical carcinogens. The mouse mammary tumor classification scheme developed by Dr Thelma Dunn and her colleagues has been widely used by veterinary pathologists and mammary biologists since its introduction in 1958 (Dunn, 1959; Sass and Dunn, 1979). It has the charm of regarding the mammary masses found in mice as ‘tumors’ without necessarily designating them as benign or malignant. The tumors were given a letter designation that was initially based on frequency of occurrence: Type A, Type B and Type C. Later, additional letters were added to signify new or additional morphologically distinct tumor types. For example, the pregnancy dependent ‘tumor’, also known as, ‘plaque’, became Type P (Dunn, 1959; Sass and Dunn, 1979).

Subsequently, Rehm and Liebelt proposed a tissue-based classification of the murine mammary lesions with a primary subdivision into alveolar, ductal and myoepithelial origin (Rehm and Liebelt, 1996). While this biologically based classification was a considerable improvement, neither the Dunn nor the tissue origin classification describe the greater variety of mammary tumors that are seen in GEM models. The recognition of novel tumor types emerging in GEMS has necessitated the development of a new classification scheme that would encompass these tumors.

The classification of human breast cancer has a time-honored, clinically validated tradition that is widely accepted and applied around the world (Jensen *et al*., 1993; Page *et al*., 1998). Descriptive terms such as medullary, cribriform, and others are understood and universally accepted. Unfortunately, the classification always distinguished between 'ductal' and 'lobular'. This terminology is maintained, however, because of long-standing use and the knowledge that 'lobular' carcinoma and 'lobular' carcinoma *in situ* have morphologic patterns and clinical outcomes that are distinct from their 'ductal' counterparts. Most authorities now agree that both types of tumors arise in the Terminal Ductal Lobular Unit (TDLU) (Cardiff, 1998; Cardiff and Munn, 1998; Cardiff and Wellings, 1999; Russo *et al*., 1990; Wellings *et al*., 1976). That is, they arise in the terminal ductules or alveoli, rather than in the collecting ducts as implied by the term 'ductal'. So while the designations are useful, they give a misleading impression about the cellular origin of the tumor. This resolution of the controversy around ductal and lobular does not exclude the possibility that other human and murine tumors actually arise in the major ducts.

The mammary gland is anatomically divided into collecting ducts and the TDLU. The TDLU terminate in alveolar buds that share a common terminal ductule (see drawings in Figure 1) (Russo *et al*., 1990). As will become apparent, the development of an anatomically correct classification requires knowledge of the anatomy of the mouse mammary gland.

At birth the female mouse mammary gland parenchyma consists of a single primary main lactiferous duct that branches into three to five secondary ducts. From the second to the fifth weeks of life, continuous branching and sprouting leads to new ducts that vary in width and length. Some of the ducts are narrow and straight, ending in club shaped terminal end buds (TEBs) (Figure 1a). This period of mammary gland development is identified as the 'ductal stage'. At the beginning of ovarian function and during sexual maturity, branching continues by budding of 'lateral buds' that end in terminal ducts (TDs), or cleavage of

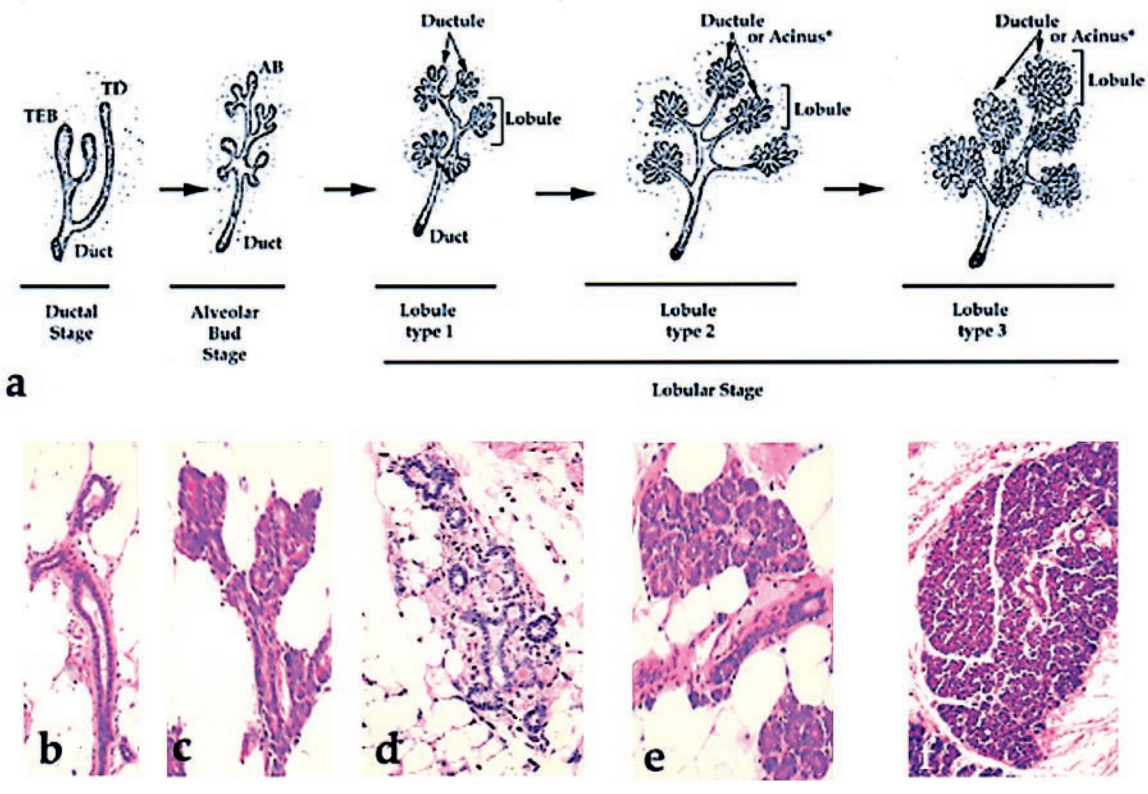

Figure 1 From the second to the fifth weeks of life, during the 'ductal stage', the mammary tree grows by continuous branching and sprouting of new, ending in club shaped terminal end buds (TEBs) (**a**). The 'alveolar bud stage' is characterized by budding of 'lateral buds' that end in terminal ducts (TDs), or cleavage of TEBs into two smaller buds, the 'alveolar buds (ABs)'. (**a,c**). The ABs sprout new buds, referred to as 'ductules' that cluster around the duct, forming a primitive lobule type 1 (Lob 1) signaling the beginning of the 'lobular stage'. The primitive Lob 1 is composed of 6.26 + 4.10 ductules per cross section (**a,d**) and with increasing stimuli form more complex lobular structures such as the lobules type 2 (Lob 2) and type 3 (Lob 3). Lob 2 contain 22 – 32 ductules per cross section (mean 26.64 + 5.22) (**a,e**). Lob 3 contain 53 – 90 ductules per cross section (mean 69.50 + 13.59) (**f**). All the lobular ductules are associated with a duct, the intralobular terminal duct. As the ductules present in Lob 2 and Lob 3 start accumulating secretory material within the lumen they are called acini or alveoli. The mouse mammary lobular structures are surrounded mainly by fat and very small amounts of connective tissue (**b – f**) (drawings and images provided by Dr Jose Russo)

TEBs into two smaller buds, the 'alveolar buds' (ABs). This stage is called the 'alveolar bud stage' (Figure 1a,c). The ABs, in turn, sprout new buds that are called 'ductules' at this stage. They cluster around the duct, forming a primitive lobular structure or lobule type 1 (Lob 1). The formation of Lob 1 signals the beginning of the 'lobular stage' that progresses from the more primitive Lob 1, composed of 6.26 + 4.10 ductules per cross section (Figure 1a,d), to more complex lobular structures such as the lobules type 2 (Lob 2) and type 3 (Lob 3). This development takes place during pregnancy or under hormonal stimulation. Lob 2 contain 22–32 ductules per cross section (mean 26.64 + 5.22) (Figure 1a,e). Individual ductules are small, lined by a layer of cuboidal epithelium surrounded by myoepithelial cells. The centrally located lumen is small and devoid of secretion. Lob 3 contain 53–90 ductules per cross section (mean 69.50 + 13.59) (Figure 1f). All the ductules of the lobular structures drain into a common duct, called intralobular terminal duct. Ductules present in Lob 2 and Lob 3 accumulating secretory material within the lumen are called acini. The epithelial cells lining the lumen of the acini have vacuolated cytoplasm due to their content of lipids. During lactation, the acini become distended with milk. The distended acini become tightly packed and the boundaries between different lobules disappear. The individual lobules become very difficult to identify. At this point in the lactating gland, the term Lob 3 is no longer applicable.

The mammary lobules of mice differ from the lobules found in the human. The lobule in humans is embedded in a relatively loose connective tissue stroma that is surrounded by a denser connective tissue (Russo *et al*., 1990). A clear demarcation between the intra- and interlobular spaces exists. In contrast, the lobular structures of the mouse mammary gland are surrounded mainly by fat and very small amount of connective tissue (Figure 1b–f) (Cardiff and Wellings, 1999; Russo *et al*., 1990). Further, type 2 and type 3 lobules are found in the resting breasts of non-lactating, non-pregnant adult human females while the quiescent mammary gland of the mouse rarely has the more developed type 1 and type 2 lobules (Russo *et al*., 1990).

Classification of genetically engineered mouse (GEM) mammary lesions

For many of the GEM models examined, the only pathological material that was available for examination was the tumor itself. In the absence of adjacent mammary tissue, tissue from other mammary glands and mammary tissue at earlier stages of tumorigenesis, the Panel was generally unable to assess the natural history of the disease. Furthermore, the existing literature on these models frequently failed to adequately describe neoplastic progression in the system. Faced with these problems of incomplete clinical descriptions of the disease, the Panel found it difficult, and perhaps even misleading, to provide a diagnostic classification that had biological significance. As a result, the Pathology Panel highly recommends the use of a descriptive classification of mammary lesions. The intent here is to correct the inadequacies of previous classifications and hold the classification open for modification as more detailed biological studies become available that clearly document the natural history of the disease in each model system.

The available evidence and our experience suggest that tumors arising in many GEM have morphological patterns that have seldom been observed in non-GEM virus-induced or carcinogen-induced mouse mammary tumors (Table 2) (Figures 2 and 3). From this perspective, the histopathology of GEM mammary tumors is unique. GEM tumors have three notable phenotypes: (1) some transgenes produce tumors closely resembling non-GEM tumors (Figure 2); (2) many transgenes produce tumors that have unique, transgene-specific phenotypes (signature tumors) (Figure 4); and (3) some GEM produce tumors that mimic human breast cancer (Cardiff and Wellings, 1999). The new nomenclature is designed to accommodate all these tumor types.

Recommendations

Descriptors A series of morphological descriptors should be applied to the GEM neoplasms (Table 3).

Table 2 Comparison of classification of mammary gland proliferative lesions

Annapolis nomenclature	*Nomenclature used in this literature* (Dunn 1959; Rehm and Liebelt, 1996; Sass and Dunn, 1979)*	*Cellular origin/differentiation (Rehm and Liebelt, 1996)*
Hyperplasia, functional, without atypia	Hyperplasia	Alveolar/ductal
Acinar hyperplasia, low grade, focal, non-GEM	Hyperplastic alveolar nodule (HAN)	Alveolar
Solid hyperplasia, low grade, focal, hormone-induced, non-GEM	Plaque/organoid/pregnancy-dependent 'tumor' (Type P)	Ductal
Mammary intra-epithelial neoplasia (MIN)	Preneoplasia or Dysplasia	Alveolar/ductal
Adenoma/carcinoma*	Adenocarcinoma	Alveolar
glandular/Acinar	Type A	Alveolar
Cribriform	Type AB/Type B/Type L	Alveolar
Papillary	Type B/Type Y	Alveolar
Solid	Type B/Type P	Alveolar/ductal
Adenosquamous	Adenoacanthoma/pale cell tumor/large cell tumor/adenosquamous carcinoma	Alveolar
Fibroadenoma	No reports	
Squamous carcinoma	Molluscoid tumor/intraductal squamous carcinoma	Ductal
Adenomyoepithelioma	Adenocarcinoma type C/carcinosarcoma/ mixed tumor/adenomyoepithelioma	Alveolar and/or ductal, myoepithelial possibly with cartilage or bone

*Alveolar or ductal origin/differentiation

These descriptors can be modified by appropriate terms (Table 4) when the biological potential, topographical distribution, cytological or histological patterns, cytological grades, inducers (etiology) and clinical context are known. With rigorous application, this morphology-based nomenclature will permit the development of accurate descriptions that can eventually be correlated with the natural history of the disease in each of the models.

The descriptors supplied in Table 3 include most of the patterns observed by the Panel in mouse mammary tumors (Figure 3). The descriptors can be

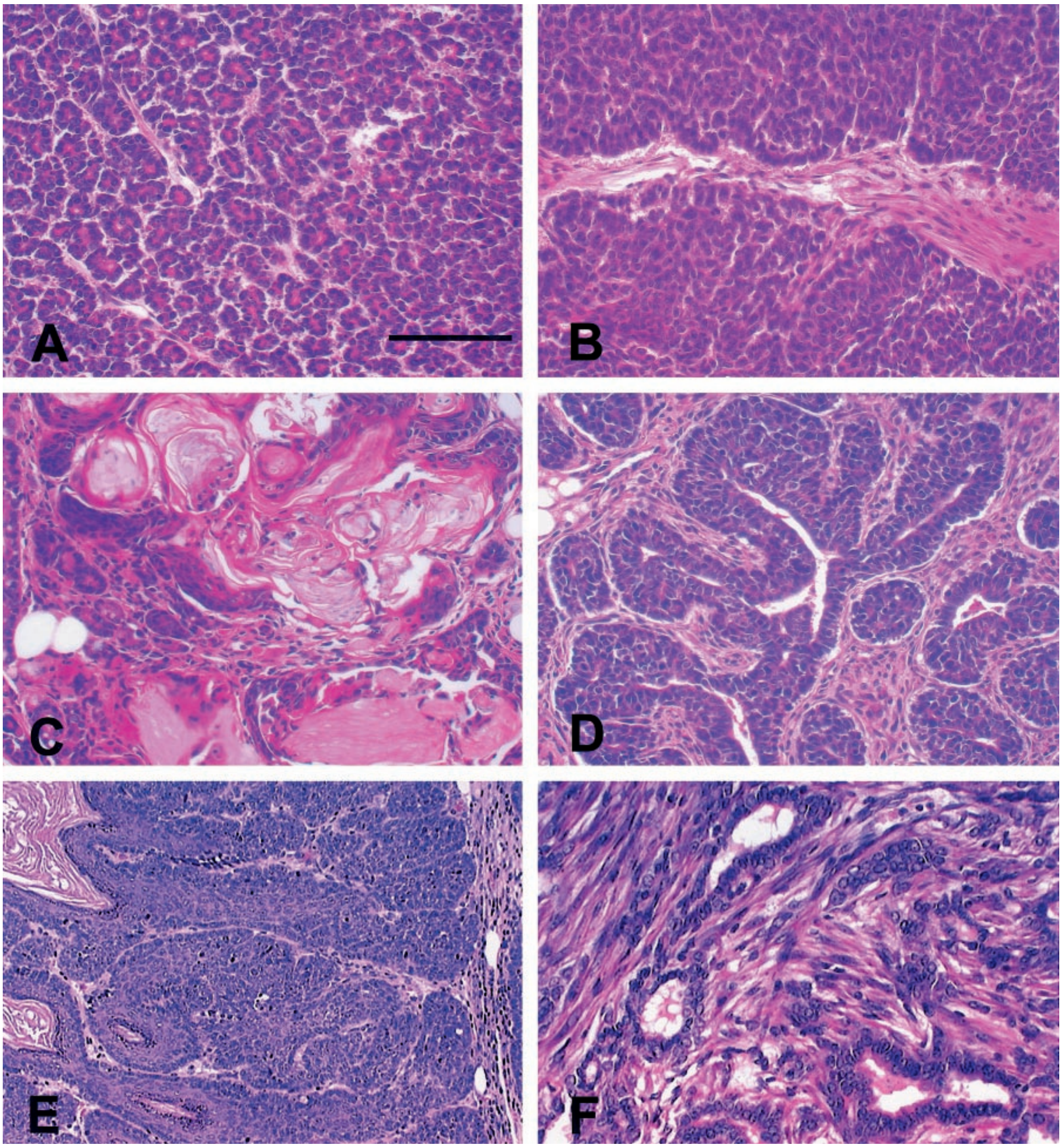

Figure 2 Photoimages showing MMTV-infected mouse mammary tumors and several other patterns associated with tumors of non-GEM mice. (**a**) The acinar pattern typical of MMTV-infected FVB mice. It is composed of small clusters of cells organized around a small lumen. The nuclei are relatively small, oval and regular. This tumor was previously classified as type A and as an acinar carcinoma, low grade, MMTV-induced in the new classification. (scale bar = 100 microns). (**b**) The solid, cord-like pattern of MMTV-infected mouse tumor. It is composed of solid cords of cells with little gland formation. The nuclei are relatively small, oval and but generally more pleomorphic that the acinar pattern tumor. It was previously classified as a type B tumor and as a solid carcinoma, low grade, MMTV-induced in the new classification. (Use scale bar in a) (**c**) The mixture of neoplastic glands and stratified squamous epithelium producing keratin. This tumor occasionally occurs in non-infected mouse strains such as FVB and more frequently in carcinogen-treated mice. This tumor is classified as an adenosquamous carcinoma. This specific tumor appeared in a transgenic MMTV-LTR/*KGF* mouse and illustrates the similarities of some GEM tumors with non-GEM tumors. (Use scale bar in a). (**d**) The combination of proliferating ducts and connective tissue was previous described as a Type P lesion. This lesion is found in mice that express the endogenous MMTV-2 and is associated with insertional activation of int-2. This lesion also appears in both the MMTV-LTR/*int-2* and MMTV-LTR/*KGF* mice. This example is from a MMTV-LTR/*KGF* mouse and would be classified as a solid MIN. (Use scale bar in (**a**)). (**e**) A chemically induced pure squamous cell carcinoma of the mammary gland with no glandular differentiation formerly referred to as molluscoid tumor. Note that the squamous epithelium is producing acellular lamellar keratin. (Taken with 20 × objective) (Photograph courtesy of Sabine Rehm). (**f**) A chemically induced adenomyoepithelioloma composed of proliferating myoepithelium and glands formerly referred to as type C tumor. (Taken with 40 × objective) (Photograph courtesy of Sabine Rehm)

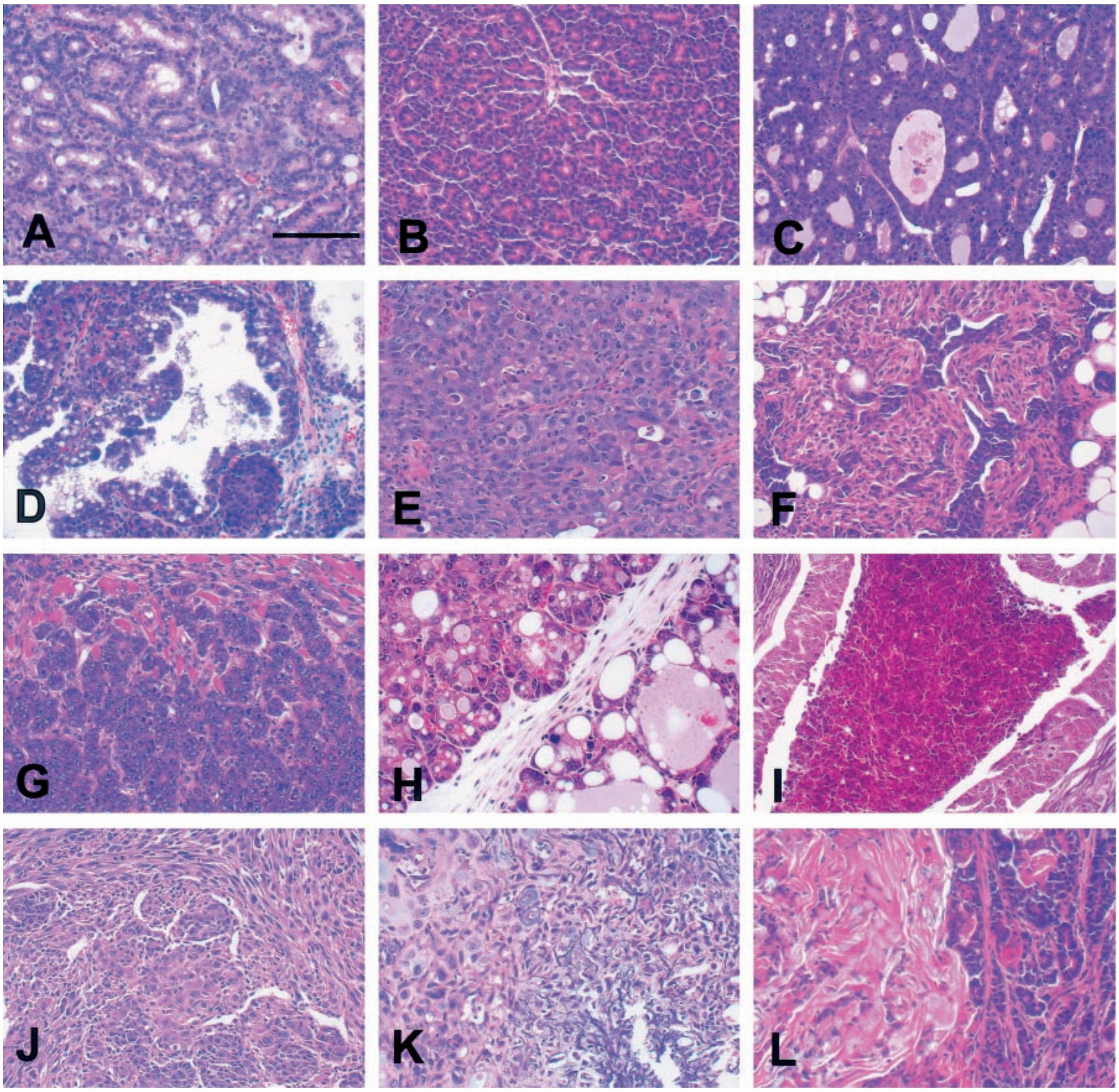

Figure 3 Examples illustrating the various descriptors and modifiers used in Tables 2 and 3 to characterize the patterns observed in GEM. (**a**) is glandular with glands dilated by secretions. This image is from a WAP/*TGFα* tumor; (**b**) is acinar with small glandular structure with small lumens and minimal secretions. The image is from a MMTV-LTR/*wnt10b* tumor; (**c**) is cribriform with back-to-back glands. The image is from a MMTV-LTR/*myc* tumor; (**d**) is papillary well differentiated with finger-like projects of fibrovascular stroma covered with neoplastic epithelium. This image is from a MTV-LTR/*TGFα* mouse; (**e**) is solid with sheets of tumor cells with little or no intervening stroma. This image is from a MMTV/*DNIIR* tumor; (**f**) is sclerotic with a dense connective tissue stroma separating strands of invasive tumor cells. This image is from a MMTV-LTR/int-3 tumor; (**g**) shows invasion between muscle fibers in a bigenic WAP/*TGFα* × WAP/*myc* tumor; (**h**) shows an expansile non-invasive pattern at the edge of an adenoma and lactating mammary gland in a bigenic WAP/p53 – 172 × WAP/*TGFα* mouse; (**i**) has necrosis at the center of a dilated glandular structure. This image is from a BLG/*igf-2* tumor; (**j**) is a carcinosarcoma tumor with sarcomatous and carcinomatous components from a WAP/stromelysin-1 mouse; (**k**) is a mammary tumor with cartilaginous and osseous metaplasia from a WAP/*stromelysin-1* mouse; (**l**) is an adenocarcinoma from a MMTV/*heregulin mouse*. The scale bar indicates 100 microns (**a**)

expanded as new morphological patterns are observed in GEM mammary lesions. However, new descriptors should avoid adaptation of terms from human surgical pathology that have clinically or biologically proven outcomes unless the investigators have shown that the biology of the lesion is also similar to the corresponding human lesion. This will avoid the continued use of conceptually inaccurate terms and misnomers.

The scientific community should avoid many of the terms traditionally used in veterinary and medical surgical pathology of the mammary gland that have little or no biological significance. For example, in surgical pathology, the words ductal and lobular in relationship to pre-malignant and malignant lesions are a now widely recognized misnomer since both neoplasms primarily originate from the terminal duct lobular unit (TDLU) (Cardiff and Wellings, 1999; Russo *et al*., 1990; Wellings *et al*., 1976). Only when the origin of a GEM tumor is actually documented should the terms ductal, ductular and alveolar be applied.

Modifiers The descriptors may be modified by a number of terms that provide additional conceptual information about the descriptors. The first set of modifiers includes terms that imply knowledge of the biological behavior of the lesion. The Panel recommends that the use of rigorous criteria be applied to each level of biological potential.

Biological potential

Hyperplastic mammary lesions (or physiologic hyperplastic lesions) Like all epithelial tissues, the mammary gland can react to damage by an insult or to physiological stimuli. In the case of the mammary gland, physiologically mediated hyperplasia by pregnancy or lactation is diffuse. Chronic exposure to endogenous or exogenous prolactin often leads to a diffuse reactive hyperplasia (Huseby *et al*., 1985). A hyperplastic repair process can occur in response to epithelial damage. This hyperplasia can be due to inflammatory conditions that may be immune-mediated, infectious or traumatic and lead to

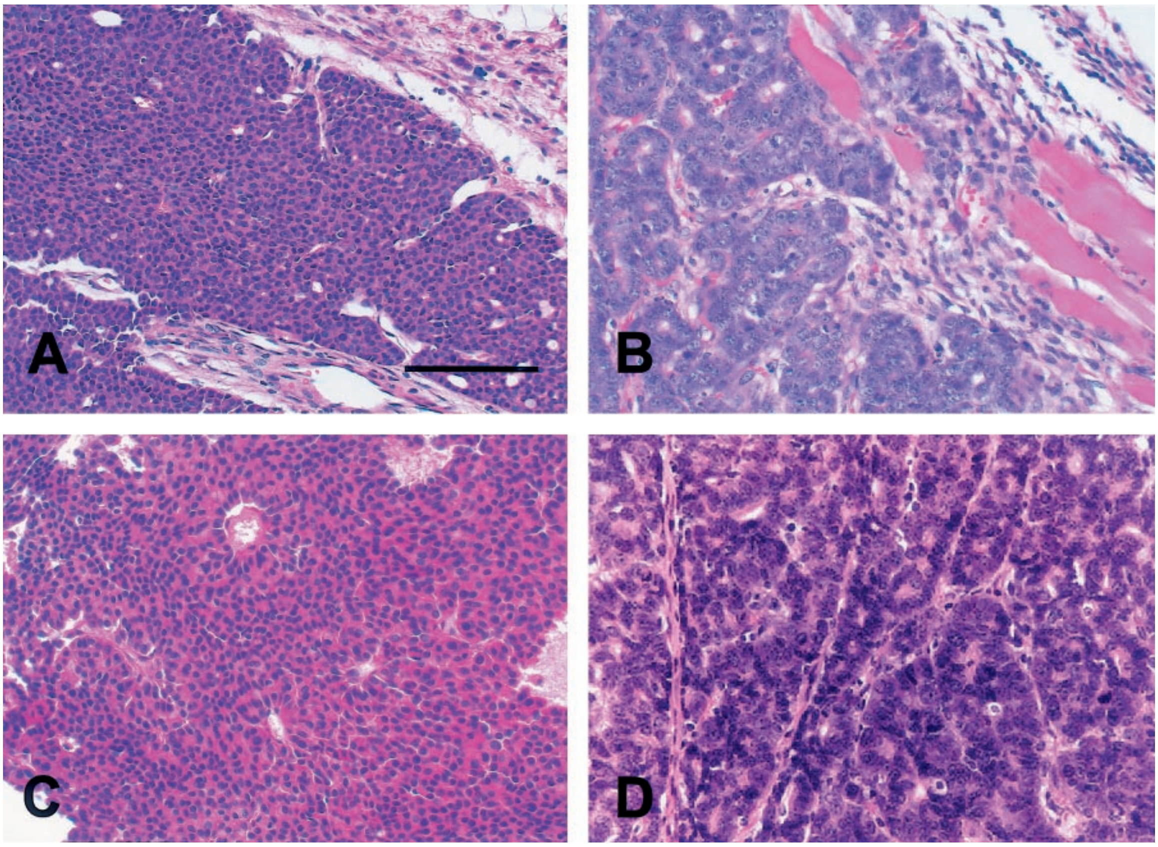

Figure 4 Comparisons between the typical phenotypes for *erb*B (*neu*) (**a**), *myc* (**b**), *ras* (**c**), and *ret*-1 (**d**). The *erb*B-type tumor is a solid, nodular tumor type with slightly atypical nuclei that are intermediate in size between the large *myc*-type cells (**b**) and the smaller *ras*-type cells (**c**). The *myc*-type tumors have large pleomorphic nuclei with a course dark chromatin and dark, amphophilic cytoplasm. These tumors are very aggressive with invasive growth patterns. This tumor is a glandular adenocarcinoma, high grade. The *ras*-type tumors have small, uniform nuclei without significant pleomorphism with relatively abundant cytoplasm. The *ras*-type tumors tend to organize around blood vessels to form papillary type growth patterns. This tumor would be classified as a papillary carcinoma, low grade. The *ret*-1-type tumor forms distinctive glands that are lined by large cells with very pleomorphic nuclei and relatively little cytoplasm and would be classified as a glandular adenocarcinoma, high grade. The scale bar indicates 100 microns (**a**)

Table 3 Descriptors

Descriptor	*Definition*	*Example*
Glandular	Tumor is composed of glands	Figure 3a
Acinar	Tumor is composed of small glandular clusters with small lumens. While this is a subclass of glandular, it is very characteristic of MMTV-induced tumors	Figure 3b
Cribriform	Tumor is composed of sheets or nests of cells forming lumens with round, punched out spaces	Figure 3c
Papillary	Tumor has finger-like projections composed of epithelium covering a central fibrovascular core	Figure 3d
Solid	Tumor is composed of solid sheets of epithelial cells with little or no glandular differentiation	Figure 3e
Squamous	Tumors composed solely of squamous cells with or without keratinization, absence of glandular pattern	Figure 2e
Fibroadenoma	Tumor is composed of a proliferation of both myxoid fibrous stroma and glands	Figure 3g
Adenomyoepithelioma	Tumor is composed of myoepithelium and glands	Figure 2f
Adenosquamous	Tumor has both glandular and squamous differentiation	Figure 2c
Not Otherwise Specified (NOS)	Tumor does not have any of the other common descriptor patterns	

Table 4 Modifiers

Modifiers	*Definition*	*Example*
Biological potential:		
Carcinoma	Neoplasm originating from epithelium with proven malignant biological behavior	Figures 2a,d,f
Adenocarcinoma	Neoplasm originating from glandular epithelium with proven malignant biological behavior	Figures 2a,3a,c
Adenoma	Neoplasm originating from glandular epithelium without proven malignant biological behavior	Figure 3h
Mammary Intraepithelial neoplasia (MIN)	Spectrum of intraluminal epithelial proliferations with cytologic atypica including *in situ* carcinomas	Figure 5
Hyperplasia	Any increase in cell number without cytologic atypia	
Tumor	Any space occupying mass with unknown biological potential	
Property:		
Atypia	Cells with abnormal nuclear morphology	Figure 5
Necrosis	Cell death generally not applied to programmed cell death (apoptosis)	Figure 3i
Fibrosis	Increased or abnormal deposition of connective tissue	Figure 3f
Secretory	Tissues or glands producing and exporting lipid or protein	Figure 3g
Metaplasia	A change from one adult cell type to another adult cell type	Figures 3k,l
Topographic:		
Diffuse	All of the mammary gland is involved in the process	
Focal	One area of the mammary gland is involved in the process	
Multifocal	Multiple foci are in the mammary gland	
Inducer (Etiology):		
Gene-induced	Tumors that have morphological or cytological patterns characteristic of specific transgenes or specific mutations. (*myc*-type, *ras*-type, *erb* B2- type)	Figure 4
MMTV-induced	Tumors known to be induced by the mouse mammary tumor virus (MMTV)	Figure 2
Chemically-induced	Tumors known to be induced by a chemical carcinogen	Figure 2e,f
Hormone-induced	Tumors known to be induced by exogenous hormones	
Biological/Experimental context:		
Biological	Parity, pregnancy, lactation, involution, hormones	
Experimental	Promotor, exogenous hormones or chemicals	

multifocal or focal hyperplasia characterized by an orderly increase in the numbers of regenerative epithelial cells within the gland. In the mouse mammary gland, these repair processes are often accompanied by squamous metaplasia of the epithelium. The inflammatory or squamous nodules frequently found in the involuted glands of multiparous females are examples. The basement membrane of the secretory unit or duct is intact but the alveolar development is persistent with additional cell layers and lymphoid infiltrates or fibrosis of the stroma. It is worth noting that many GEM models require hormonal stimulation (e.g. through continuous breeding or the application of pituitary isografts) in order to get high level of transgene expression. Thus, a physiological hyperplasia may be expected in many of these models systems, and information about hormonal manipulation of the system should be made available to the pathologist.

Precancerous mammary lesions (atypical hyperplasia, mammary intraepithelial neoplasia and carcinoma in situ) All cancers in humans and animals probably arise from single cells (Cardiff, 1984; Morris and Cardiff, 1987). In specific tissues, however, the origin of cancer can be multicentric. A sequence of molecular events leads to a sequence of histological and histogenetic changes that produces an invasive carcinoma (Cardiff, 1984). The earliest visible histological changes in the both human and murine mammary glands are focal or multifocal hyperplasias primarily within the terminal ductule or alveolar bud or both (Cardiff and Wellings, 1999). While some growths may extend into the major ducts, few lesions appear to originate in the collecting ducts as is implied by the word 'duct' or 'ductal' (Figure 5).

Preneoplasia in non-GEM mice Two types of precancerous lesions occur in MMTV-infected mice, the hyperplastic alveolar nodules (HAN) and the plaque (Cardiff, 1984; Morris *et al*., 1990). The HAN is a focal alveolar hyperplasia that stands out from the background of the non-lactating mammary gland as flat, 1–2 mm nodules. The HAN histologically resemble normal prelactating mammary gland but are clonal lesions with an experimentally proven, highly malignant potential. They are typically cytologic low-grade lesions with relatively normal nuclei but with increased DNA synthesis and mitotic rate.

The plaques are virally induced lesions and start as circumscribed ductal proliferations that are pregnancy dependent and regress on parturition. The plaque appears during pregnancy as a flat but palpable lesion with radiating ducts and a delicate connective tissue stroma. Following multiple pregnancies some of these lesions will continue to grow after the pregnancy has ended to form invasive carcinomas that differ in morphology from the hyperplastic lesions. Both lesions have interchangeably been referred to as plaque or type P tumor and this confuses the issue. Like the HAN, plaques are clonal proliferations that can progress to malignancy with hormone independence and invasion (Morris *et al*., 1990). They also have relatively low-grade cytology.

MIN in GEM The spectrum of lesions analogous to hyperplasias with or without atypia and *in situ* carcinomas in humans, has rarely been observed

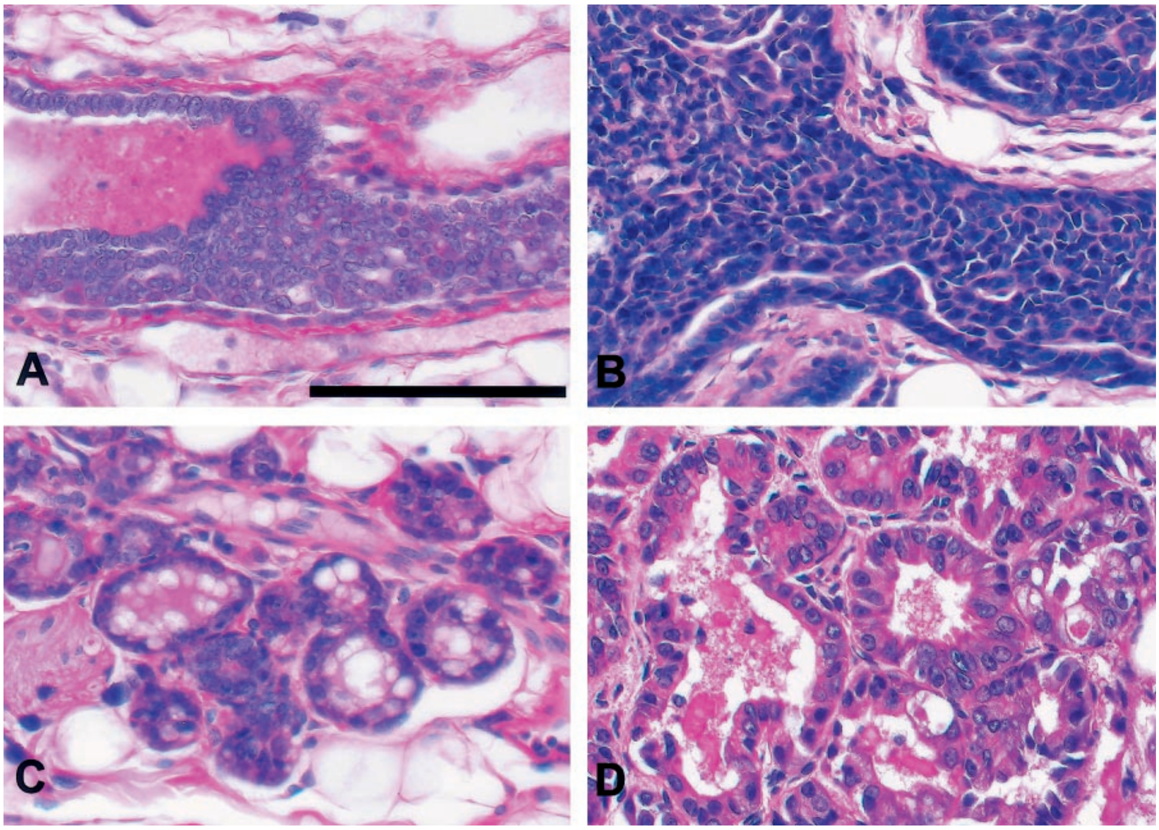

Figure 5 Illustrating low-grade (**a**,**c**) and high-grade (**b**,**d**) lesions of mammary intraepithelial neoplasms (MIN) found in the ducts (**a, b**) and in the terminal ductules (**c**,**d**). Note the relative degree of nuclear pleomorphism and hyperchromasia. (**a**) is from a MMTV-LTR/*int-3* mouse. (**b**) is from a C(3)1/*SV40* tag mouse. Figure (**c**) is from a MT/*HGF* mouse. (**d**) is from a WAP/*TGFα* mouse. The scale bar indicates 100 microns (**a**)

within collecting ducts, terminal ducts or alveoli of mice until the advent of GEM (Figure 5). Because the lesions described below appear to represent precursors to invasive carcinoma in the mammary gland and, in at least some models, have been shown to possess at least some of the molecular changes seen in invasive carcinomas (Shibata *et al*., 1996), the term 'mammary intraepithelial neoplasia' (MIN) is to be used for these lesions (Tavassoli, 1998).

The reader should note that the definition of MIN recommended for the mouse mammary gland differs from that provided by Tavassoli for the human breast (Tavassoli, 1998). The definition of MIN in the human breast, according to Tavassoli, includes hyperplasias without atypia (Tavassoli, 1998). The MIN without atypia is, in part, an interpretation based on molecular evidence. However, similar evidence from GEM mammary hyperplasias is not available. Therefore, the consensus of the Annapolis Pathology Panel is that the use of MIN in mice should be limited to strict morphological criteria including atypical nuclear cytology. As the transplant and molecular biology of the focal hyperplasias with or without atypia is documented, the definitions can be altered.

MMTV-LTR/*Int2*(*FGF3*) and MMTV-LTR/KGF(*FGF8*) models were particularly instructive in that they produced signature lesions that resembled the pattern seen in hormone dependent lesions (plaques or Type P tumors) of GR3/A mice. These lesions form circumscribed complex radiating ducts in dense connective tissue and are associated with insertional activation of *FGF3* by MMTV (Morris *et al*., 1990) (Figure 2d). Therefore, over-expression of the oncogene resulted in the same morphological lesion whether induced by the virus or inserted as a transgene. Of particular note is the production of these proliferative ductal lesions in GEM models has allowed the study of their evolution. When induced by a transgene, the lesions are irreversible, multifocal and benign. The invasive neoplasms that emerge from these focal lesions have a phenotype distinctly different from the precursor lesion and metastasize. Thus, these ductal hyperplasias are MIN and cannot be considered malignancies.

The majority of the MIN lesions observed to this point clearly originate in the TDLU. However, this could be related to the promotor used to generate the mice. The majority of current model systems use the MMTV long terminal repeat promotor/enhancer (MMTV-LTR) or the whey acidic protein promoter (WAP) that appear to produce alveolar lesions. However, in some models, such as C(3)1-promoted *SV40 Tag* (Figure 5b), the earliest lesions appear to originate in the both the collecting ducts and the terminal ductules. In at least several other GEM, for example in MMTV/ or WAP/*notch4* and MMTV-LTR/*DIINR*, the collecting ducts may have primary involvement, but further study of natural history in these models will be required to determine their origin. At this point in time, more thorough study of the earliest lesions using a combination of transplantation of the specific lesion and whole mounts and histology of the whole mammary gland are required to define the origin of these lesions.

Until these studies are completed and the biology understood, the Panel recommends a simplified classification for MIN. In this simplified classification, the MIN lesions are to be classified as low or high grade (Figure 5). The low grade lesions are characterized by the presence of hyperchromatic duct, luminal and/or myoepithelial cells with little cytoplasm, more than one layer of atypical cells, intact basement membrane and an increased mitotic rate (Figure 5a,c). High-grade lesions show more layers of epithelium, pleomorphism of nuclei and/or epithelial cells and/or an increase in mitotic figures (Figure 5b,d). Filling of the lumen by these proliferative cells can occur but the basement membrane remains intact.

If the hyperplasia produces a palpable nodule with no histological evidence of a breach in the basement membrane, a diagnosis of adenoma is appropriate. If disruption of the basement membrane is seen with malignant appearing cells traversing the area of the former basement membrane, invasive carcinoma is diagnosed. In some cases, special stains such as PAS or anti-collagen IV might be required to demonstrate the loss of basement membrane.

The term MIN infers that such lesions are not tumors, neither adenomas nor carcinomas, but rather morphological intermediates between normal and cancer. They are putative preinvasive lesions with characteristics of both benign and malignant cells. While they are probably immortalized and probably not reversible, little research has been done to support this concept. They are thought to be essentially biologically identical to similar lesions seen in other conventional mouse mammary pre-cancers such as the HAN. The MIN in GEM frequently resembles many lesions in human epithelial tissues prior to or accompanying invasive carcinoma. MIN should, therefore, be considered neither as benign nor malignant lesions but rather a preneoplastic, protoneoplastic or premalignant state.

Adenomas differ from either MIN or carcinoma in that they are well-differentiated local tumors that do not progress to malignancy. They have no malignant potential. When transplanted, they have limited local growth potential. In contrast, MIN lesions have cytological and histological atypical morphology and are thought to have malignant potential. Although the malignant potential has been amply demonstrated in non-GEM precancers, similar studies have not been systematically carried out in GEM-related MIN.

The terms dysplasia, atypia or atypical hyperplasia have also been used for lesions described above. We discourage the use of these terms for synonymous lesions but recognize that they are used synonymously for our newly described MIN.

Malignant neoplasia The most neutral term for a neoplasm is tumor. The term simply implies a space occupying mass without known biological potential. However, it is generally applied only to masses that are considered neoplastic. Tumor should be applied to all biologically uncharacterized masses thought to be neoplastic but without proven invasion or proven metastasis. The mammary glands of mice frequently have non-invasive, low-grade neoplasms without evidence of metastases. These large focal tumors without known metastases or without proven invasion are considered adenomas. Only when invasion or metastasis is observed can the modifier carcinoma or adenocarcinoma be justified.

Criteria for malignancy The malignant potential can be judged by three criteria: (1) the histological demonstration of invasive behavior; (2) demonstration that these tumors actually metastasized; or (3) experimental assessment of biological potential by transplantation of the mass.

The biological potential can best be evaluated on the basis of biological observation and experimentation. The time-honored operational criteria in the mouse mammary biology are: (1) identification of the lesion *in-situ*; (2) isolation of the lesions by surgical extirpation; (3) transplantation into a gland free mammary fat pad and into the subcutaneous fat of syngeneic or immunocompromised hosts (Cardiff, 1984; Medina, 1996; Morris *et al*., 1990). Transplants of normal mammary gland will result in outgrowth of a normal mammary tree in the fat pad but no growth in the subcutaneous tissue. The normal mammary outgrowth will survive for only a limited number of 3 – 5 transplantions. Premalignant hyperplastic mammary lesions will fill the mammary fat pad with hyperplastic outgrowths that are immortal. They can be serially transplanted indefinitely but will not grow as subcutaneous transplants. Their malignant potential can be judged by the number of malignant tumors that arise secondarily from the outgrowth. In contrast, lesions that are completely transformed will grow as either subcutaneous or mammary fat pad transplants that may or may not metastasize but will continue to grow in either site.

Morphological criteria for malignancy can also be applied. Acceptable criteria include invasive growth patterns (Figure 3g) or metastasis. Many GEM and non-GEM tumors appear to be more expansile than invasive and, frequently, have a relatively low-grade cytology (Figure 3h). The histological demonstration of malignant potential requires adequate sampling of the tissue surrounding the tumor and harvesting of potential metastatic sites, particularly the lungs. However, caution must be exercised in correlating tumor phenotype with metastases. Many, if not most, transgenic mice have multifocal mammary tumors. Without experimental verification, it may be impossible to prove that the metastasis came from any one of the many mammary tumors.

In a like manner, caution must be used when applying molecular criteria for neoplasia. Non-GEM premalignant mouse mammary hyperplasias are clonal and frequently have loss of heterozygosity (Cardiff, 1984; Marchetti *et al*., 1995; Morris *et al*., 1990). Even lesions with a low risk of malignant progression have these molecular characteristics. Since the individual lesions can be isolated and transplanted for comprehensive study of their natural history, the GEM are excellent experimental models for the testing of molecular concepts of neoplastic progression.

Grading Histological and cytological grading has proven useful in assessing the biological potential of human breast cancer (Charpin *et al*., 1995; Genestie *et al*., 1998; Le Doussal *et al*., 1989). The grading systems attempt to establish standard criteria for histological organization, mitotic rate and nuclear atypia. All MIN and neoplasms should be graded to determine which cytological criteria correlate with outcome.

The low-grade lesions are characterized by the presence of well-organized glandular patterns, hyperchromatic nuclei without significant pleomorphism but with reduced cytoplasm. The mitotic rate is increased (Figures 2a,b and 3h). High-grade lesions have less differentiated glands, more nuclear pleomorphism and a higher mitotic rate (Figure 3c,f,g).

In general, the GEM models have much higher grade tumors that in the non-GEM, spontaneous tumors in mice. Unfortunately, the cytological grade in GEM does not always correspond with the rate of metastasis (Cheung *et al*., 1997). However the GEM models provide an excellent opportunity to determine which factors are the most prognostically significant.

Property and topography

Although the categories of property and topography are self explanatory, several aspects need emphasis. The properties are not diagnostically significant terms but may be used to modify the diagnosis, providing ancillary information about the tumor. They should not be used in isolation but only to modify the primary diagnosis. The topographical data is significant in evaluating the biology of the lesion. The modifier 'diffuse' means that all of the mammary gland is involved, implying a universal or systemic phenomenon. If only portions of the gland are involved, the word multifocal is more appropriate. The use of multifocal or focal in relation to proliferating cells suggests a neoplastic event. These modifiers should be included in descriptions of any lesion so that the data can be collected for further study.

Inducer (etiology)

GEM models are being used to evaluate the role of various genes in mammary cancer. With increasing frequency, the GEM models are being experimentally manipulated. Experimental introduction of exogenous hormones, exogenous viruses, carcinogens and other modifiers is designed to change the natural history of the disease. The morphology will also change and the experimental inducer must be explicitly noted. Some factors are discussed below.

Non-GEM mouse tumors

Three types of lesions are typical of MMTV-induced tumors (Figure 2). Veterinary pathologists have traditionally described them as acinar, tubular or solid. These MMTV-induced lesions have sufficiently unique characteristics and their origin is known that they should be recognized and acknowledged by all pathologists. Interestingly, many carcinogen-induced tumors are either adenosquamous or undifferentiated but additional tumor types occur such as adenomyoepitheliomas (Rehm, 1990). Again, the patterns and origins are well known. However, the morphology of chemically induced tumors may depend on the strain and cell of origin (Rehm, 1990).

Mammary tumors in GEM

MMTV-type tumors Interestingly and reassuringly, GEM models generated with oncogenes originally identified by MMTV insertional mutagenesis developed tumors that closely resembled those induced by the MMTV viruses. The MMTV-LTR/*int2*(*FGF3*), wnt1/*int2*, *notch 4* and *wnt10b* transgenes produced MMTV-type tumors. MMTV-LTR promoted transgenes (*wnt1* and *wnt10b*) produced tumors more closely resembling the acinar tumors (Figures 2 and 3).

It is important to note that many other transgenic animals also produced sporadic tumors with squamous metaplasia (adenosquamous carcinoma or adenoacanthoma) (Figure 2c). This tumor phenotype is also frequently observed in association with chemical carcinogenesis in BALB/c and C3H strains (Medina, 1976, 1988; Medina and Warner, 1976). The mammary epithelium of older mice under chronic prolactin stimulation commonly has squamous metaplasia (Huseby *et al*., 1985). It is important when interpreting tumors with squamous metaplasia in GEM mice to consider the possibility that these tumors are not due to the genetic manipulation, but are sporadic tumors. It must be remembered that factors that induce squamous metaplasia in other mice can also be operative in GEM mice.

GEM signature tumors Several of the models present in the Annapolis slide set or from the Panel's private archives, had tumors with unique phenotypes. Such signature phenotypes were observed in association with myc, c-*erb*B2, *ras*, and *ret*1 (Figure 4). The best example was probably the *myc*-type tumors that have relatively large cells with large, pleomorphic nuclei with a course chromatin and prominent nucleoli. The cytoplasm also tended to relatively abundant and darkly amphophilic. This signature *myc* phenotype was seen under the influence of different promotors, in tumors with different grades of differentiation and in combination with different secondary transgenes (compare Figures 3c,h and 4b). In contrast, the c-*erb*B-2 tumors have much more uniform, smaller nuclei with a delicate chromatin and abundant pink cytoplasm (Figure 4a). The *erb*B2 tumors tend to grow as solid expansile nodules of cells. Ras associated tumors have a smaller more regular nucleus and abundant reddish cytoplasm (Figure 4c). The *ras* tumors tend to grow as solid papillary lesions resembling transitional cells of the urinary bladder. The *ret1* tumors formed small crowded glands with large pleomorphic nuclei and scanty reddish cytoplasm (Figure 4d). The unique characteristics of each transgenic tumor type are very significant because they suggest that tumor phenotype can be correlated with the molecular alteration in mice and perhaps in human (see below) (Cardiff *et al*., 1991).

Some 'signature' tumor phenotypes have also been observed in some human breast cancers. For example, the so-called ductal carcinoma *in situ* (DCIS) of the comedo type has a distinctive morphological atypia and central necrosis. This form of DCIS generally overexpresses c-*erb*B2 (Barnes, 1993). More recently, morphological features which distinguish between BRCA1- and BRCA2- associated human tumors have been described (Lakhani *et al*., 1998). Some special types of human breast cancer appear to be associated with particular genetic alterations (Armes *et al*., 1998), suggesting that human tumor phenotype is also heavily influenced by genotype. We expect that additional phenotypes will be correlated with genotype leading to a molecular classification for both human and murine breast cancers. However, whether the same 'signature' phenotype will be induced by a given genetic alteration in the different genetic backgrounds of human and mouse is not yet clear.

It should be emphasized that the unique GEM-induced phenotypes are signature tumors that are found solely or primarily associated with a particular transgene. Tumors with other, less characteristic, phenotypes do also appear in association with the same transgene at varying frequencies (Cardiff *et al*., 1991). A signature tumor type appears to be occasionally induced by an entirely different transgene. These exceptions could indicate the recruitment or overexpression of different ancillary genes. The mammary biologist studying any given model system must be alert to appearance of these non-signature tumors.

GEM tumors that mimic human tumors Finally, some of GEM models contained lesions that closely resembled human breast neoplasms (Cardiff and Wellings, 1999). Although the similarities were impressive, it should be emphasized that they tended to be represented in restricted foci of tumors and not necessarily the entire tumor. As a result, an experienced surgical pathologist would confuse few mouse tumors with human lesions. The closest resemblance was found amongst the more poorly differentiated phenotypes. However, transgenes such as c-*erb*B2 produced a range of lesions that resembled solid or comedo forms of so-called DCIS of humans (Cardiff and Wellings, 1999). SV-40 Tag also produced DCIS type lesions (Shibata *et al*., 1996). Examples of *notch4*-induced lesions produced a unique ductal atypia that mimics DIN or atypical hyperplasia (Tavassoli, 1997, 1998; Tavassoli and Norris, 1990). Papillary carcinomas that could mimic human cancer were seen in many of the transgenic models such as MMTV-LTR/*cyclin* D1, *met1* and BGL/*IGF2* (Cardiff and Wellings, 1999). Lastly, tumors with regions densely sclerotic stroma can be found associated with the *myc*, PyV-mT, SV-40 Tag, WAP/*int-3* and *src* transgenes (Figure 3f,g) (Cardiff & Wellings, 1999).

Multi-genic GEM tumors A number of GEM tumors that develop in animals with two or more engineered genes have been described. These bigenic or trigenic animals produce tumors that are neither mixtures of the signature tumor phenotype nor a unique phenotype (Cardiff, 1995, 1991; Cardiff and Munn, 1995). Rather, tumors in multigenetic mice tend to express the phenotype of the dominant transgene. For example, the *myc* transgene is dominant in combination with transgenes such as *ras*, *erb*B2, *BCL2*, *TGFa*, and *heregulin* (Cardiff *et al*., 1991). All mice with the *myc* transgene, independent of promotor system, develop mammary tumors that have *myc*-type cytology (Figures 3c and 4b). As might be expected, when a transgene is coupled with mice with targeted mutations of tumor suppressor genes, the tumors always resemble the transgenic signature pattern. In some targeted mutations such as *p53*, the overall pattern of the transgene is retained but with a tendency towards higher cytological grades with increasing nuclear pleomorphism and aneuploidy.

Biological/experimental context

The pathologic interpretation of GEM mammary lesions requires knowledge of the biological and experimental context in which the lesions developed. In a hormone sensitive organ such as the mammary gland factors such as parity, pregnancy, lactation, involution and endogenous or exogenous hormones are extremely important (Russo *et al*., 1990). The information should accompany all samples submitted to the pathologist. Preferably, the pathologist should be consulted in advance and be involved and informed about the experimental design and sampling times.

The promotor system used to create the GEM is particularly important and should be recorded as an important part of the diagnostic information. The promotor in many GEM models influences the phenotype. For example, the pattern observed with *TGF*α is quite different in the WAP based systems as compared to the MMTV-LTR based systems (Figure 3i,d,g,h). On the other hand, *myc* is such a dominant transgene that its phenotype appears to be independent of the promotor (Figure 3c,g). The promotor, in some cases, appears to effect the cell of origin. For example, C(3)1 appears to be associated with lesions in the major collecting ducts while MMTV-LTR appears to be more likely associated with lesions with a TDLU origin (Shibata *et al*., 1996). These conclusions are based on a very limited set of samples but emphasize the need to record the promotors.

Ancillary tests and immunophenotyping

The Panel repeatedly sought the types of additional information provided by ancillary tests such as *in situ* hybridization, image analysis and immunophenotyping. This type of information has proven to be extremely valuable in the understanding and interpretation of human breast diseases. Some of this information is now incorporated into the routine assessment of human breast cancers. However, the literature has very few examples of systematic or comparative immunophenotyping of tumors in GEM model systems. As a result, the Panel could not be provided with information that it considered important in its interpretation of the biology and natural history of the disease.

Immunocytochemical techniques have proven useful in documenting critical biological events in human breast cancer. The Panel, therefore, recommends that all mammary models be characterized by immunohistochemistry and/or *in situ* hybridization studies. Table 5 provides the categories that should be studied and some of the recommended markers where available.

Pathobiology

Our understanding of the pathology of human breast cancer is based on the correlation of each morphological entity with molecular and clinical-pathological parameters. Because large numbers of cases of individual types of human breast cancer have been studied within the context of clinical trials, it has been possible to produce predictive information on likely survival and metastatic potential for an individual case. The major difficulty that faced the Panel was insufficient information to predict the natural history of the disease. The vast majority of animals with MIN lesions had no accompanying data to predict the likely behavior and potential progression of the lesions. In order to identify the most appropriate models for extensive molecular analysis, further and more detailed studies of their natural history must be done. An experienced pathologist who is part of the research team must study the pathology of these animals over a prolonged time course. The investigator is obliged to collect the appropriate information so that patterns of morphological progression and biological potential can be established.

Experimental, clinical and pathological information is required for interpretation of the mammary pathobiology. The Panel has created report forms for the biologist and the pathologist that feature the key data points and descriptors (see Appendix). These forms are designed to guide both teams in the recording of data. The collection of this data is essential. The principal investigators should concentrate on collecting observations that lead to an understanding of the natural history of the disease.

Mouse models afford an excellent opportunity to study and understand the evolution of breast disease. More attention needs to be given to the time course of the growth and development of the mammary gland in these model systems. Many model systems develop early abnormalities that, in and of themselves, provide insight into mammary biology. When coupled with neoplastic development, these insights will lead to advances in the understanding and treatment of human breast cancer. For example, mammary whole mount and histological specimens should be taken from GEM during development at 4, 8, 12 and 14 weeks after birth and pregnancy, lactation and involution. Age and parity matched control mice from the investigator's own colony are essential for adequate interpretation.

The starting and terminal points of tumor progression deserve particular attention. On a purely cytological basis, the Panel approves of the term *MIN* to denote intraluminal proliferations of epithelium that have significant nuclear atypia. However, this concept must be validated by a combination of tissue transplantation to prove the biological potential of such atypical lesions and by molecular analysis to demonstrate properties such as LOH and clonality associated with other such lesions. The role of these techniques in assessing the biology has been described previously.

Many of the mammary tumors in the slide set were relatively low grade and typically had expansile or pushing margins rather than outright invasion. By most criteria, such lesions are intraepithelial neoplasms, non-malignant neoplasms or adenomas. However, the veterinary community is very familiar with such tumors resulting in pulmonary metastases and properly regards them as malignant tumors. Since such information is not always available in the GEM model systems, it needs to be systematically collected. The necropsy prosector needs to be carefully observant of the lung and other potential sites of metastasis. The pathologist needs to search more carefully for evidence of vascular invasion and microinvasion of the stroma.

Finally, the research community needs to remember that many tumor cells form emboli that may appear in the lung but do not colonize the lung. This distinction is critical. The investigator should not count small intravascular collections of cells as 'pulmonary metastasis'. Many of the small tumor emboli are only transient in the lung and will not colonize the lung. We recommend that only those foci that expand the lumen of the blood vessel or invade the wall of the blood vessel be considered as pulmonary metastasis.

Table 5 Phenotypic markers

Transgene expression	IHC or ISH
Nuclear receptors	ER, PR
Myoepithelium	Smooth Muscle Actin
Luminal cell	Cytokeratin, Muc-1, casein, lactalbumin, lactoglobin
Basement membrane	Laminin, collagen IV, PAS
Proliferation (S phase)/Apoptosis	Ki67/Tunel assays
Other	Vascular markers (CD31 [PECAM], SMA), neuroendocrine, p53

Pathologic technique

The quality of many of the samples found in the Annapolis slide set suggested to the Panel that some investigators need guidance in the specimen sampling, fixation and processing. An outline of recommended procedures for the necropsy of animals and for specimen fixation and processing is appended. These recommendations are reflected in the forms that are also appended.

Several aspects of specimen procurement and processing need emphasis. First is the need for harvesting of the 'normal' host tissue adjacent to the lesion. While the molecular analysis of tumors requires samples without normal tissue, the pathologic analysis requires the interface between the host and its tumor. The interface between the two contains valuable information about the growth characteristics of the tumor (invasive versus expansile) and the reaction of the host to its tumor. Further, the adjacent mammary tissue often contains atypical regions that give important insight into neoplastic progression and the mammary pathobiology associated with the specific gene. On excision of the tumor, cut widely around the mass and leave all surrounding tissues attached including the overlying skin, adipose subcutaneous tissues and underlying musculature. On trimming the mass, be sure to include attached representative sections of normal adjacent tissues.

Inadequate fixation appears to be a common problem. The quality of fixation on some specimen slides suggest either that the sample was not exposed to the fixative long enough to permeate the entire mass, the fixative had exceeded its shelf life or the ratio of fixative to tissue was inadequate. In some cases, the pathologist cannot accurately assess the preparations. In others, poor fixation precludes immunohistochemistry or *in situ* hybridization.

The investigator should also note that the precise procedures for sampling and processing would depend on the research design. Some antigens are destroyed or masked by specific fixatives. Standard formalin fixation does not provide as good nuclear detail as metal or acidic based fixatives. Alcohol is preferred for nucleic acid extraction or *in situ* hybridization but creates shrinkage artifacts. Once the specimen is fixed, it can be 'post-fixed' to recover some antigens or cytological detail. For example, antigens and nuclear detail in formalin-fixed tissue can be recovered by post-fixation in mercury based fixatives such as B5. Investigators are encouraged to incorporate an experienced pathologist into their research team so that such details can be worked out in advance of harvesting of the samples.

Finally, while most investigators are using the whole mount technique, apparently some groups do not routinely use whole mounts to evaluate their animals. This simple technique allows survey of the entire mammary structure and should be systematically used on all studies of rodent mammary biology.

The pathologist

As indicated above, investigators who wish to have an accurate assessment of their model need to incorporate an experienced pathologist into their team throughout the course of the research program. Pathology is the study of disease. Pathology is not simply the naming of histological patterns. As discussed throughout this essay, accurate interpretation of the disease process requires a thorough knowledge of the structure and function of the mammary gland, the context in which the disease occurs, its natural history, and etiology. A tumor is not a cancer just because someone declares it so. It is only diagnosed as a cancer when morphologic evaluation is coupled to careful studies of the natural history of the disease. The pathologist is the person trained to assist the investigator in integrating this information.

The introduction of genetic engineering into mouse pathology has provided a unique and exciting new biology. The Annapolis Pathology Panel was composed of a cross section of expertise in experimental, surgical and veterinary pathology. This panel of experts often found the mouse models presented were far beyond their experience and, therefore, difficult to interpret. Our recommendations are based on our combined experience and expertise. We suspect that no single pathologist has such a combination of experts readily available and is at risk of misinterpreting slides from genetically engineered animals. The Panel, therefore, recommends that a panel be developed to review slides from various NCI grantees. The panel will help educate the local institutional pathologist by sharing their expertise and bring a more standard nomenclature to transgenic pathology. The panel can be modeled after those that have been so successfully used by the organized cooperative oncology groups in the United States and Europe. The scientific community will benefit through the training of local pathologists and the rapid recognition of the unique histopathological aspects of their animals. The inclusion of surgical, experimental and veterinary pathologists on such a national panel will enhance the study of comparative pathology of model systems.

Comparative pathology of the model systems

The Annapolis slide set documented that GEM produce categories of mammary tumors that are not seen in MMTV-induced or other experimentally induced mouse mammary tumors. Some of the tumors have patterns that mimic the histopathology of human breast lesions in great detail. These intriguing lesions merit much more intense study as they promise to unlock the molecular mysteries currently facing us in human breast cancer. In fact, all of the genes represented in the slide set and other models merit further study because they all inform us of the normal and abnormal biology of the mammary gland.

The National Cancer Institute has challenged the scientific community to develop a molecular classification of human tumors. The mouse mammary biologists have already started the marriage between pathology and molecular biology of cancer by creating animals with known genetic abnormalities that have distinctive morphological lesions. As we learn the rules of abnormal structure and function from the mouse, we should apply them to human breast cancer.

One needs to be mindful that the mouse is a different species. No matter how exciting the similarities, there are differences between murine and human mammary cancer. Some of the more obvious or common similarities and differences are listed in Table 6.

The Panel was encouraged to learn that the molecular biologists already have plans to address

Table 6 Comparison of human and mouse mammary biology

Similarities	*Differences*
Molecular lesions causing breast cancer in humans have proven to cause breast cancer in GEM	Some molecular lesions causing breast cancer in mice have not been found in human breast cancer
Similar morphological patterns of lesions appear in both species	The morphology of most mouse tumors does not resemble the common human breast cancers
Development of cancer consistent with multi-hit kinetics	Some transgenes appear to be associated with one-hit kinetics
Breast cancers in both species are metastatic	Most mouse tumors metastasize to the lung. Most human tumors metastasize to the regional lymph nodes
Both species have distinctive host responses to the cancers	The mouse generally does not respond to its tumor with as much fibrosis and inflammation
Breast cancer is frequently hormone independent	Half of the human breast cancers are hormone independent. Most mouse tumors are hormone independent

Table 7 Summary of pathology workup

Tissue	*Fix*	*Block*	*Section*	*Analysis*
Mammary tissue				
1. Mammary masses, including adjacent uninvolved mammary tissue where possible, or contralateral mammary gland*	x	x		Diagnosis and molecular analysis
2. Representative mammary glands**: Thoracic (#2/3 or #7/8) and inguinal (#5 or #10)	x	x	Longitudinally ('fried egg' morphology)	Preneoplastic changes
3. Remaining mammary glands	x			More extensive survey or preneoplastic changes, if required later
Organs to analyse for metastases				
1 Lung	x	x		Metastases
2. Lymph nodes (axillary and inguinal)***	x	x		Metastases
3. Liver	x	x		Metastases
4. Femur (including marrow)	x		Longitudinal	Metastases
5. Vertebrae, with spinal cord	x		cross-section cervical, thoracic, lumbar	Metastases
6. Brain	x		Half, sagittal	Metastases
Other hormonally sensitive organs				
1. Uterus, both ovaries	x	x		Changes in other hormonally sen-sitive organs
2. Pituitary	x	x		Pituitary changes that might affect MG
The rest				
1. Other gross lesions	x	x		Additional effects of experimental intervention
2. Rest of the mouse, including e.g. head skin with ears	x			To allow other organs to be worked up later if necessary, and for confirmation of animal ID

*For masses >0.5 cm diameter, snap freeze half for molecular analysis and fix remaining half. Separately freeze some uninvolved tissue (can use contralateral gland). **In all mice, including those without gross lesions, take at least two mammary glands routinely. Be consistent about always taking the same gland. If the chosen gland has a lesion, harvest the contralateral gland. (The adjacent thoracic glands 2/3 or 7/8 can be harvested together). ***The axillary and inguinal lymph nodes can each be separately grouped with other tissues when blocked, for ease of identification and to reduce the number of blocks

many of these differences by creating new types of promotors, constructs and transgenes. While no one murine model system may represent the complete model system, increasing the biological and morphological similarities to human cancer will be afforded by careful attention to specific scientific questions raised in the study of clinical breast cancer.

The Annapolis Meeting has reinforced the need of the scientific community to optimize their communication. Towards this end, the Panel and organizers have posted over 800 annotated images concerning the comparative pathology of GEM and human breast cancer at URL: *http://histology.nih.gov/*. In addition, a comprehensive table showing the entire slide set is available at the same URL. We are in the process of developing an interactive CD-ROM to introduce scientists and students to the pathobiology of the mouse mammary gland. This program will be available through and have appropriate educational hot links to the resources on the mammary site at NIH. We hope that this resource will serve to quickly close the gap between morphologic and molecular biology.

Summary of recommendations

1. An interim descriptive morphology-based nomenclature be applied to mammary lesions in mice until sufficient knowledge of their natural history allows a biology based classification system.
2. The development of a 'clinical' classification be deferred until the biology of the atypical intraluminal lesions is more adequately understood.
3. Detailed comparative studies using ancillary techniques such as immunohistochemistry and *in situ* hybridization be undertaken to establish relationships between human and murine cancer.
4. Standard protocols for the sampling, collection, processing, and reporting of specimens be adopted by the scientific community to ensure more uniform and thorough analysis.
5. Funding agencies should strongly encourage that research teams studying animal models of disease include an experienced pathologist with an interest in mammary biology and pathology to ensure careful morphologic evaluation.
6. NIH should establish a panel of experts for review of murine mammary pathobiology so that the necessary degree of experience can be developed and disseminated.

Acknowledgements
The Annapolis meeting was sponsored by the NIH Breast Cancer Think Tank and the NCI Cancer Genome Anatomy Project. The organizers and panelists would like to thank Ms Susan Greenhut for her assistance in organizing the meeting, slides, and logistics for the meetings. We would also like to thank Jai Evans and

Ulrike Wagner for their excellent technical assistance throughout the course of this project. The organizers and panelists gratefully acknowledge the following investigators who graciously provided information, blocks and slides to the slide sets: Ari Elson, Priscilla Furth, Jeffrey Green, Hans Weiher, Glenn Merlino, A Graessmann, Izumi Nakashima, Chris Graham, Daniel Medina, Glenn Radice, Eugene Lukanidin, Mark Sternlicht, Barbara Knowles, Dimitrina Pravtcheva, Gilbert Smith, Sonia Jakowlew, Eric Sandgren, Barry Davis, Lothar Hennighausen, Robert Cardiff, Ann Harrington, Jake T Liang, Emmett Schmidt, Philip Leder, Harold Varmus, Robert Coffey, Rob Callahan, Bill Muller, Roy Jensen, Jeffrey Rosen, Hal Moses and Jose Russo. This project has been funded in part with Federal funds from the National Cancer Institute, NIH, under contract no. NO1-CO-56000 and a grant from CGAP.

References

Armes JE, Egan AJ, Southey MC, Dite GS, McCredie MR, Giles GG, Hopper JL and Venter DJ. (1998). *Cancer,* **83,** 2335–2345.
Barnes DM. (1993). *J. Cell. Biochem.* Suppl., **17G,** 132–138.
Bates P, Fisher R, Ward A, Richardson L, Hill DJ and Graham CF. (1995). *Br. J. Cancer,* **72,** 1189–1193.
Cardiff RD. (1984). *Adv. Cancer Res.,* **42,** 167–190.
Cardiff RD. (1995). *J. Mammary Gland Biology and Neoplasia,* **1,** 61–73.
Cardiff RD. (1998). *J. Mammary Gland Biology and Neoplasia,* **3,** 3–5.
Cardiff RD and Munn RJ. (1995). *Cancer Lett.,* **90,** 13–19.
Cardiff RD and Munn RJ. (1998). *Breast Cancer: Advances in Oncobiology*, Vol. 2. Heppner G (ed.). JAI Press Inc., pp.177–202.
Cardiff RD, Sinn E, Muller W and Leder P. (1991). *Am. J. Pathol.,* **139,** 495–501.
Cardiff RD and Wellings SR. (1999). *J. Mammary Gland Biol. Neoplasia,* **4,** 105–122.
Charpin C, Bacquie N, Bouvier C, Devictor B, Boulat J, Andrac L, Lavaut MN, Allasia C and Piana L. (1995). *Anticancer Res.,* **15,** 2611–2617.
Cheung ATW, Young LJT, Chen PCY, Chao CY, Ndoye A, Barry PA, Muller WJ and Cardiff RD. (1997). *Int. J. Oncology,* **11,** 69–77.
Coffey RJ, Jr., Meise KS, Matsui Y, Hogan BL, Dempsey PJ and Halter SA. (1994). *Cancer Res.,* **54,** 1678–1683.
Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS and Bradley A. (1992). *Nature,* **356,** 215–221.
Dunn TB. (1959). The physiopathology of cancer, Vol. 2. F, H (ed.). Hoeber: New York, pp.38–84.
Furth PA, BarPeld U, Li MG, Lewis A Laucirica R, Jager R, Weiher H and Russell RG. (1999). *Oncogene,* **18,** 6589–6596.
Gallahan D, Jhappan C, Robinson G, Hennighausen L, Sharp R, Kordon E, Callahan R, Merlino G and Smith GH. (1996). *Cancer Res.,* **56,** 1775–1785.
Genestie C, Zafrani B, Asselain B, Fourquet A, Rozan S, Validire P, Vincent-Salomon A and Sastre-Garau X. (1998). *Anticancer Res.,* **18,** 571–576.
Guy CT, Cardiff RD and Muller WJ. (1992a). *Mol. Cell. Biol.,* **12,** 954–961.
Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD and Muller WJ. (1992b). *Proc. Natl. Acad. Sci. USA.,* **89,** 10578–10582.
Huseby RA, Soares MJ and Talamantes F. (1985). *Endocrinology,* **116,** 1440–1448.
Husler MR, Kotopoulis KA, Sundberg JP, Tennent BJ, Kunig SV and Knowles BB. (1998). *Transgenic. Res.,* **7,** 253–263.
Iwamoto T, Takahashi M, Ito M, Hamaguchi M, Isobe K, Misawa N, Asai J, Yoshida T and Nakashima I. (1990). *Oncogene,* **5,** 535–542.
Jager R, Herzer U, Schenkel J and Weiher H. (1997). *Oncogene,* **15,** 1787–1795.
Jensen RA, Dupont WD and Page DL. (1993). *J. Cell. Biochem.* Suppl., **17G,** 59–64.
Jhappan C, Stahle C, Harkins RN, Fausto N, Smith GH and Merlino GT. (1990). *Cell,* **61,** 1137–1146.
Joseph H, Gorska AE, Sohn P, Moses HL and Serra R. (1999). *Mol. Biol. Cell.,* **10,** 1221–1234.
Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G and Smith GH. (1995). *Dev. Biol.,* **168,** 47–61.
Lakhani SR, Jacquemier J, Sloane JP, Gusterson BA, Anderson TJ, van de Vijver MJ, Farid LM, Venter D, Antoniou A, Storfer-Isser A, Smyth E, Steel CM, Haites N, Scott RJ, Goldgar D, Neuhausen S, Daly PA, Ormiston W, McManus R, Scherneck S, Ponder BA, Ford D, Peto J, Stoppa-Lyonnet D, Easton DF *et al.* (1998). *J. Natl. Cancer. Inst.,* **90,** 1138–1145.
Lane TF and Leder P. (1997). *Oncogene,* **15,** 2133–2144.
Le Doussal V, Tubiana-Hulin M, Friedman S, Hacene K, Spyratos F and Brunet M. (1989). *Cancer,* **64,** 1914–1921.
Li B, Rosen JM, McMenamin-Balano J, Muller WJ and Perkins AS. (1997). *Mol. Cell. Biol.,* **17,** 3155–3163.
Liang TJ, Reid AE, Xavier R, Cardiff RD and Wang TC. (1996). *J. Clin. Invest.,* **97,** 2872–2877.
Kitsberg DI and Leder P. (1996). *Oncogene,* **13,** 2507–2515.
Krane IM and Leder P. (1996). *Oncogene,* **12,** 1781–1788.
Kwan H, Pecenka V, Tsukamoto A, Parslow TG, Guzman R, Lin TP, Muller WJ, Lee FS, Leder P and Varmus HE. (1992). *Mol. Cell. Biol.,* **12,** 147–154.
Marchetti A, Buttitta F, Miyazaki S, Gallahan D, Smith GH and Callahan R. (1995). *J. Virol.,* **69,** 1932–1938.
Maroulakou IG, Anver M, Garrett L and Green JE. (1994). *Proc. Natl. Acad. Sci. USA.,* **91,** 11236–11240.
Matsui Y, Halter SA, Holt JT, Hogan BL and Coffey RJ. (1990). *Cell,* **61,** 1147–1155.
Medina D. (1976). *Cancer. Res.,* **36,** 2589–2595.
Medina D. (1988). *Carcinogenesis,* **9,** 1113–1119.
Medina D. (1996). *Cancer. Treat. Res.,* **83,** 37–69.
Medina D and Warner MR. (1976). *J. Natl. Cancer. Inst.,* **57,** 331–337.
Morris DW, Barry PA, Bradshaw HD, Jr. and Cardiff RD. (1990). *J. Virol.,* **64,** 1794–1802.
Morris DW and Cardiff RD. (1987). *Adv. in Viral. Oncology,* **7,** pp.123–140.
Muller WJ, Sinn E, Pattengale PK, Wallace R and Leder P. (1988). *Cell,* **54,** 105–115.
Muller WJ, Lee FS, Dickson C, Peters G, Pattengale P and Leder P. (1990). *EMBO J.,* **9,** 907–913.
Page DL, Jensen RA and Simpson JF. (1998). *Breast Cancer Res. Treat.,* **51,** 195–208.
Pravtcheva DD and Wise TL. (1998). *J. Exp. Zool.,* **281,** 43–57.
Rehm S. (1990). *Am. J. Pathol.,* **136,** 575–584.
Rehm S and Liebelt AG. (1996). *Pathobiology of the Aging Mouse*, Vol. 2. Mohr U, Dungworth DL, Capen CC, Carlton WW, Sundberg JP and Ward JM. (eds). ILSI Press: Washington DC, pp.381–398.
Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR and van Zwieten MJ. (1990). *Lab. Invest.,* **62,** 244–278.
Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL and Lee DC. (1990). *Cell,* **61,** 1121–1135.

Sandgren EP, Schroeder JA, Qui TH, Palmiter RD, Brinster RL and Lee DC. (1995). *Cancer. Res.*, **55,** 3915–3927.
Sass B and Dunn TB. (1979). *J. Natl. Cancer. Inst.*, **62,** 1287–1293.
Sinn E, Muller W, Pattengale P, Tepler I, Wallace R and Leder P. (1987). *Cell,* **49,** 465–475.
Shibata MA, Maroulakou IG, Jorcyk CL, Gold LG, Ward JM, Green JE. (1996). *Cancer Res,* **56,** 2998–3003.
Smith GH, Gallahan D, Diella F, Jhappan C, Merlino G and Callahan R. (1995). *Cell. Growth. Differ.*, **6,** 563–577.
Stewart TA, Pattengale PK and Leder P. (1984). *Cell,* **38,** 627–637.
Takayama H, LaRochelle WJ, Sharp R, Otsuka T, Kriebel P, Anver M, Aaronson SA and Merlino G. (1997). *Proc. Natl. Acad. Sci. USA.*, **94,** 701–706.
Tavassoli FA. (1997). *The Breast. Journal,* **3,** 48–58.
Tavassoli FA. (1998). *Mod. Pathol.*, **11,** 140–154.
Tavassoli FA and Norris HJ. (1990). *Cancer,* **65,** 518–529.
Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T and Varmus HE. (1988). *Cell,* **55,** 619–625.
Tzeng YJ, Gottlob K, Santarelli R and Graessmann A. (1996). *FEBS. Lett.*, **380,** 215–218.
Tzeng YJ, Guhl E, Graessmann M and Graessmann A. (1993). *Oncogene,* **8,** 1965–1971.
Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A and Schmidt EV. (1994). *Nature,* **369,** 669–671.
Wellings SR, Jensen HM and DeVault MR. (1976). *Experientia,* **32,** 1463–1465.

Annapolis Pathology Request Form for Studies of the Mouse Mammary Gland

Principal Investigator :________________Institution:__________________

Tissue Submitted By:________________________________ Date: _______/______/______

Animal Number:________ Strain: _______________ Age:__________(Weeks/Mo)

Experimental: __________ Control: ______ Carcinogen:__________ Virus:________________

Specify Promotor(s): __________________ Specify Transgene(s):__________Genotype:______/_______

Gender: M______F____ No of Pregnancies: ___________ Days of Gestation/Lactation:__________

Date tumor first noted: _______/______/______Date of Sacrifice:_______/______/______

Fixative: ______________ Days of Fixation: _______

Processing: Routine (yes/no) ASAP(extra charge): (yes/no) Date: _________/______/_______

Tissue Collected: (yes/no) Tissue Fixed: (yes/no) Tissue Frozen: (yes/no)

			Yes	No			Yes	No
Mammary Gland Sampled:								
	1.	Left Cervical	☐	☐	6.	Right Cervical	☐	☐
	2.	Left Thoracic	☐	☐	7.	Right Thoracic	☐	☐
	3.	Left Thoracic	☐	☐	8.	Right Thoracic	☐	☐
	4.	Left Abdominal	☐	☐	9.	Right Abdominal	☐	☐
	5.	Left Inguinal	☐	☐	10	Right Inguinal	☐	☐
Tissues Sampled:								
A) Soft tissues:		Lung	☐	☐		Liver	☐	☐
		Brain	☐	☐		Lymph Nodes	☐	☐
		Ovaries/Testis	☐	☐		Adrenal glands	☐	☐
		Pituitary	☐	☐		Kidneys	☐	☐
		Thymus	☐	☐		Spleen	☐	☐
		Other (specify):						
			Note:	*Please indicate site of lymph node sampling*				
B) Skeleton		Vertebrae	☐					
		Femur	☐					
Unsubmitted Tissue Fixed For Future Study			☐	☐				

Gross Description and Experimental Protocol

Annapolis Pathology Reporting Form:

Principal Investigator :________________Institution:__________________

Tissue Submitted By:______________________________ Date: ______/______/______

Animal Number:_______ Strain: _______________ Age:__________Weeks/Mo)

Experimental: _________ Control: ______ Carcinogen:__________ Virus:________________

Specify Promotor(s): __________________ Specify Transgene(s):__________Genotype:______/_______

Gender: M_____F___ No of Pregnancies: ____________ Days of Gestation/Lactation:__________

Fixative:______________ Days of Fixation: _______

Date Received:_________/_______/_______ Date of Processing: ____/_______/_______

Macroscopic Description of Tissue Received:

This should include the details on the request form, together with the pathologist's description before and after tissue slicing. The size of any tumor or lesion identified should be measured in the maximum diameter in mm. All abnormalities should be described.

Annapolis Pathology Reporting Form
Microscopic Description:

A) BENIGN LESIONS		**YES**	**NO**	
	Fibroadenoma	☐	☐	
	Adenomyoepithelioma	☐	☐	
	Adenoma	☐	☐	
	Nuclear atypia present	☐	☐	
	Atypia Grade	High ☐	Medium ☐	Low ☐
B) EPITHELIAL PROLIFERATION:		**YES**	**NO**	
	1. Acinar hyperplasia	☐	☐	
	- Physiological Hyperplasia increase in numbers of acini	☐	☐	
	- GEM-induced Hyperplasia Alveolar	☐	☐	
	- GEM-induced Hyperplasia Ductal	☐	☐	
	- Focal	☐	☐	
	- Multifocal	☐	☐	
	- Diffuse	☐	☐	
	2. Intra-luminal proliferation	☐	☐	
	- Involves ducts	☐	☐	
	-- terminal duct lobular units	☐	☐	
	-- Unknown	☐	☐	
	- Epithelial proliferation without atypia	☐	☐	
	- Epithelial proliferation with atypia	☐	☐	
	-- Atypia Grade	High ☐	Medium ☐	Low ☐
	- Focal	☐	☐	
	- Multifocal	☐	☐	
	- Diffuse	☐	☐	
	Note: *Epithelial proliferation with atypia is considered as Mammary Intraepithelial Neoplasia (MIN) by the Annapolis nomenclature*			
C) MALIGNANT LESIONS:		**YES**	**NO**	
	Maximum Diameters of Tumor	____ x ____		(mm)
	Tumor Pattern			
	- Glandular	☐	☐	
	- Acinar	☐	☐	
	- Cribriform	☐	☐	
	- Papillary	☐	☐	
	- Solid	☐	☐	
	- Squamous	☐	☐	
	- Other (specifiy)			
	Invasion	☐	☐	
	Vascular Invasion	☐	☐	
	Necrosis	☐	☐	
	Fibrosis	☐	☐	
	Overall Grade	1 ☐	2 ☐	3 ☐

Mammary lymph nodes		
- Number positive		
- Total		
Other node metastases present	☐	☐
- Number positive		
- Total		
Site of other nodes		

D) Metastases:

	YES	NO
1. Lung metastases	☐	☐
2. Brain metastases	☐	☐
3. Liver metastases	☐	☐
4. Bone metastases	☐	☐
5. Report on other sites examined		

E) Description of Other Microscopic Findings:

Summary of Findings:

Pathologist: ______________________________

IDEALIZED MOUSE WORKUP FOR MAMMARY TUMORIGENESIS STUDIES

The following outline is provided as a guide for maximizing the information obtained from your mouse. Since considerable information can be obtained from analysis of microscopic changes in the mammary gland, it is particularly important to give the pathologist representative "uninvolved" mammary tissue in addition to gross mammary lesions. This can give insight into the preneoplastic processes occurring in your model.

PROCEDURE:

1. For any newly generated mouse model, the founder mice and a select number of offspring (ideally no less than 5) should be subjected to a complete necropsy in order to get a complete description of the phenotype. This will allow identification of any additional phenotype changes that might impact on mammary tumorigenesis studies. A guideline for performing complete necropsies can be found at http://www.ncifcrf.gov/vetpath/necropsy.html.
2. For mice in a mammary tumorigenesis study, it is preferable to have a minimum of 10 mice/group. The use of appropriate controls is critical. There should be an equal number of age-matched, non-engineered or untreated controls, of identical genetic background and parity. Note that mammary gland morphology and the incidence of spontaneous mammary tumors varies widely between different genetic backgrounds. This is a particularly important issue when working in a hybrid background. The pathologist should be supplied with the information about the mice found in the Pathology Request Form.
3. For a mammary tumorigenesis study, the mouse should be opened and examined grossly, with any lesions or gross changes noted on the necropsy sheet. Examine all mammary glands grossly and note positions and size of lesions on a diagram of the glands at URL: http://www.ncifcrf.gov/vetpath/necropsy.html. Assign a unique post-mortem number to the mouse.
4. To maximise the amount of information obtainable from each mouse, the tissues indicated in Table 7 should be harvested for each mouse. Tissues of immediate interest should be processed to paraffin block as quickly as possible (within 48h for formalin) as this gives best results for immuno-histochemistry. Organs that are not immediately useful can be fixed and saved for later use (see #8 below). Correct identification of all mice is critical. It is useful to include the identifier (e.g. ears if mice are ear-notched) to allow subsequent cross-checking of mouse ID with PM number.
5. Mammary masses: For masses >0.5cm, it may be desirable to snap freeze half for molecular analysis and fix half for histology (see notes about fixatives below). For smaller masses, fix all for histology. If possible include some uninvolved gland, and harvest the contralateral gland for both histology and molecular analysis.
6. Whole mounts: To visualize the morphology of the ductal tree, it is desirable to whole mount some glands from a select subset of animals. The #4 and #9 (abdominal glands) are generally best for whole mounting because of the presence of the lymph node for orientation. A protocol for mammary whole mounts can be found at http://www-mp.ucdavis.edu/tgmice/Histolab.html. Lesions identified at whole mount can subsequently be sectioned for histology.
7. Trimming and sectioning mammary glands: For trimming mammary glands, remove mammary gland from skin and put on paper (rough DRY brown paper towels work well). Press gland on towel and flatten with forceps. Alternatively, the gland can be spread on a glass slide for better visualization. You can trim off some fat at this point. It is helpful to have a magnifying glass present when trimming muscle away from glands (esp. for thoracic glands). Glands are then fixed (see below).
8. Fixatives:
   a. If the study will require use of specific antibodies for immunohistochemistry, it is critical to research the optimal fixative for the antibody of interest in advance. Remember that the mammary epithelium is embedded in excessive amounts of fat that either needs to be trimmed or may require special defatting procedures.
   b. For many applications, 10% neutral buffered formalin can be used. Agitate tissues in a 10x-fold excess volume of fixative overnight at room temperature and then change to fresh neutral buffered formalin for storage. For best results with immunohistochemistry, PROCESS TO BLOCK WITHIN 48h. When saving tissues in fixative, double bag all untrimmed tissue with formalin. Recheck bags after 6 mo. and top up with formalin if necessary.
   c. For *in situ* hybridization, best results are usually obtained with 4% paraformaldehyde as fixative. Fix for 3-5d at 4C. Paraformaldehyde needs to be fresh (store at 4°C for a max of 4 days). Store fixed tissues, blocks and tissue sections at 4°C.
   d. The study and grading of nuclear atypia is better with some acidic or heavy metal fixatives (Bouin's, Zenker's, B-5 ect). Tissue stored in formalin can be post-fixed with these fixatives to improve nuclear detail.
9. Sectioning and staining. When cutting sections from mammary gland blocks, cut longitudinally ("fried egg morphology"). 5u sections are optimal. Hematoxylin/eosin staining is usually optimal for histology. A very light hematoxylin counterstain is generally used alone for immunohistochemistry. However, other nuclear counterstains may be better for specific purposes. Sometimes diagnosis will be aided by additional special stains (e.g. immunohistochemical staining for smooth muscle actin).